# Physiologic and Pathogenic Specialization of Rhynchosporium secalis

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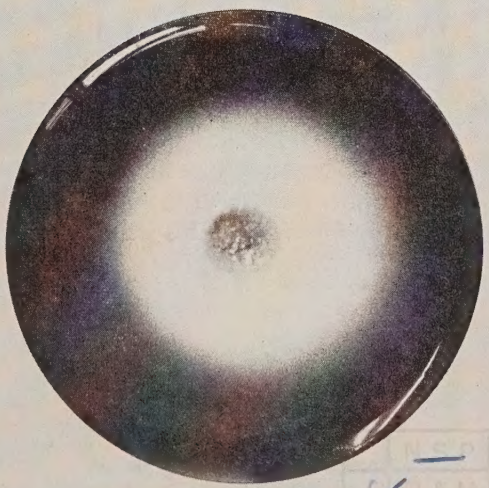

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## DIGEST

Isolates of the barley scald fungus, *Rhynchosporium secalis*, were obtained from Pennsylvania, North Carolina, Tennessee, Michigan, South Dakota, and California. In a series of tests, seven barley varieties were inoculated with each of these isolates. Seven distinct pathogenic races of the organism were thus determined. In addition, it was found that one of the isolates from California was capable of infecting several wild grasses. This raises the possibility that barley is not safe from attack by *Rhynchosporium* from wild grasses growing near commercial barley plantations.

In other studies, 991 varieties and lines from the world collection of winter barleys were tested against the seven races. As a result of this work, three varieties were found to be immune to attack while four others were found to be so infrequently attacked as to make excellent sources of resistance in breeding programs.

Studies showing the cultural variability of the organism are reported in the concluding section of this bulletin. Responses to pH, various nitrogen sources, vitamins, and carbohydrates are given. Starch digestion was accomplished by an alpha-amylase which was found to diffuse through the agar far in advance of the mycelium. This helps explain the slow, determinate growth of the organism in culture.

Acknowledgments: The author wishes to acknowledge that a large portion of this work, particularly that reported in the concluding section of this bulletin, was performed by John W. Kerelo. Assistance also was rendered by J. A. Snow. Both of these men were Graduate Assistants in Plant Pathology.

Grass seed used in these studies was supplied by Dr. A. A. Hanson, Forage and Range Branch, Crops Research Division, United States Department of Agriculture, Beltsville, Maryland.

# Physiologic and Pathogenic Specialization of Rhynchosporium secalis

Richard D. Schein†

SCALD DISEASE OF BARLEY, incited by *Rhynchosporium secalis* (Oud.) J. J. Davis, was of minor importance in Pennsylvania until the introduction of certain susceptible varieties. Wong, a very susceptible winter barley, has become the most planted variety in this State, and scald has increased in prevalence as a result.

Introduced by the New York Agricultural Experiment Station in 1941, Wong (CI 6728) has many desirable characteristics. It is winter hardy, high yielding, stiff strawed, and the grain is borne in awnleted spikes (heads). Although Hudson is scald-resistant, out-yields Wong, and is more winter hardy in Pennsylvania, its long, rough awns discourage ready adoption.

Winter barley is one of Pennsylvania's important cereal crops, about 250,000 acres being seeded annually. The grain and straw are important to the livestock industry, and much of the barley grown is used on the farms where it is produced. Because it is such a valuable feed grain, breeding of an awnleted variety less susceptible to scald than Wong and adapted to Pennsylvania conditions would be very desirable.

The objectives of the work reported herein were: (1) to screen the world collection of winter barleys for resistance to scald disease and (2) to determine whether pathogenic races of the fungus exist in the United States and whether such variation should be considered and used in the breeding program for scald resistance.

In early phases of the studies, a certain amount of cultural work was conducted by the author and by John W. Kerelo, a graduate assistant on the project. The results of these investigations, of use to scientists wishing to maintain cultures of *Rhynchosporium*, are presented in the last section of this report.

Barley scald disease and its causal agent have been under investigation in various parts of the world since about 1895. To cite all cases of field observation around the world or to review all pertinent liter-

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ature in this introductory section would be pointless. Previous studies which have yielded important information are discussed in the sections where they pertain. Materials and methods used in each experiment are presented with the results in the later sections. In this way the reader is spared the chore of constantly referring to the introductory section to determine how the work was conducted.

## I. Pathogenic Specialization

### A. Specialization of United States Isolates

The barley scald disease, incited by *Rhynchosporium secalis* (Oud.) J. J. Davis, has been the subject of several investigations in the United States. No racial specialization of isolates from barley was recognized until 1955, although it has been known since 1947 that such races exist in Argentina. The presence or absence of races must, of course, be known before a thorough breeding program can be developed. Furthermore, paradoxical conclusions have been drawn about the pathogenic specialization of this organism, particularly as to whether a specific isolate can attack members of genera other than its source host genus.

Caldwell (2) outlined the history of nomenclature and the results of early specialization studies from the first report of the organism as *Marsonia secalis* in 1897 until 1937. He emended the description to include the characteristic features of fructification. It is evident from this review that European workers could find no indications of pathogenic specialization, since isolates of the organism from such hosts as barley (*Hordeum vulgare* L.), rye (*Secale cereale* L.), or several non-cereal grasses could attack all of these plants. Caldwell's own research showed a very different situation. In cross-inoculation studies, he used 20 isolates from Wisconsin, 3 from Oregon, and 1 from Germany. He reported that isolates from barley parasitized barley and *Hordeum murinum* L.; those from foxtail barley (*Hordeum jubatum* L.) attacked *H. jubatum*, *H. nodosum* L. (possibly *H. brachyantherum* Nevski), and *H. pusillum* Nutt.; those from quackgrass (*Agropyron repens* (L.) Beauv.) attacked *A. repens* and *A. trachycaulum* (Lk.) Malte. (synonym: *A. tenerum* Vasey); those from smooth brome (*Bromus inermis* Leyss.) attacked *B. inermis*, *B. arenarius* Labill., *B. lanuginosis* Poir. (probably *B. macrostachys* Desf.), *B. madritensis* L., and *B. villosus* (probably *B. rigidus* Roth); those from Canada wild-rye (*Elymus canadensis* L.) attacked *E. canadensis*, *E. virginicus* L., and *E. striatus* (probably *E. villosus* Muhl.), as well as *Agropyron trachycaulum*. Through overinterpretation of this work, there is a widespread belief in the United States that barley is safe from attack by the *Rhynchosporium* races of Caldwell that commonly occur on non-cereal grasses.

Caldwell found no evidence that isolates of *Rhynchosporium* from barley were specialized in their ability to attack different varieties of barley. In 1947, two Argentinian workers, Sarasola and Campi (*9*), reported two findings of significance to the present study. They found that isolates of *Rhynchosporium* from barley attacked four species of grasses in four genera — *Elymus canadensis*, *Agropyron smithii* Rydb., *Festuca elatior* L., and *Bromus unioloides* H.B.K. (probably *B. catharticus* Vahl.). In addition, they reported that various isolates obtained from barley were specialized in ability to attack varieties of barley. On the basis of the reaction of three differential varieties, they characterized four races of the pathogen.

At present, the literature reports two kinds of races of *Rhynchosporium:* (1) those of Caldwell based on the ability of the fungus to attack members of different genera of plants, and (2) those of Sarasola and Campi based on the ability of the organism to attack different varieties of the same host species.

In addition to these papers, one other body of reports bears on the present investigation. Sprague (*13*) reported on grasses naturally infected by *Rhynchosporium secalis.* His list contains at least 26 species in 10 genera of the Gramineae. Finally, it should be noted that evidence of pathogenic specialization was reported earlier by the author (*10*); by Houston and Ashworth (*4*), who have found at least five races or biotypes in California since 1953; and by Reed (*8*).

**MATERIALS AND METHODS.** — A number of isolates of *Rhynchosporium secalis* were used in this study. The Pennsylvania isolate was obtained by the author. In addition, two isolates were received from California, from B. R. Houston and L. J. Ashworth, Jr.; one from Tennessee, from H. E. Reed; one from North Carolina, from J. C. Wells; two from Michigan, from R. Kiesling; and two from South Dakota, from J. Hennen. An isolate from *Hordeum vulgare* was purchased from the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands. Except for the California and North Carolina isolates, diseased leaves were supplied by the cooperators, and the author made the isolations through use of a previously published technique (*11*). All cultures were maintained as mass transfers on slants of Lima bean agar (Difco), a medium on which this fungus sporulates abundantly.

Barleys and non-cereal grasses used in these studies were grown in soil in pots or flats. In inoculation experiments, 3 to 10 plants of each variety of barley and 50 to 100 plants of the grasses were used. In some cases, only small amounts of barley seed could be obtained, and these were increased in greenhouse and field, precautions being taken to ensure self-pollination and purity. The nomenclature follows that used by Hitchcock and Chase (*3*).

Inoculations were made when the barleys reached the 3-leaf stage, 14 to 16 days after planting; the non-cereal grasses were usually 3 weeks old. Inoculum was grown during the same period; plantings and transfers usually were made the same day. Cultures were transferred with a smear technique that resulted in the production of many small colonies per slant. Conidia were harvested by adding about 5 milliliters (ml) of distilled water and rubbing the colonies off with a sterile transfer needle. The colonies were broken up with a sterile rubber policeman, and the resulting suspension of mycelium, spores, and agar was filtered through a double thickness of 24-by-20-mesh cheesecloth. Inoculation consisted of spraying the plants with the spore suspension until wet. Preliminary studies showed few if any discernible differences among symptoms on plants inoculated with a series of sprayings with suspensions containing over 50,000 spores per ml. Thereafter, to minimize dosage-difference effects, spore assays before each inoculation ascertained that at least 50,000 spores per ml of inoculum were present. A dosage range of 50,000 to 500,000 spores per ml ultimately was used. After inoculation, the plants were removed to a moist chamber maintained at 17 to 21° Centigrade for 48 hours, after which they were transferred to a greenhouse held at temperatures of 20 to 25° C.

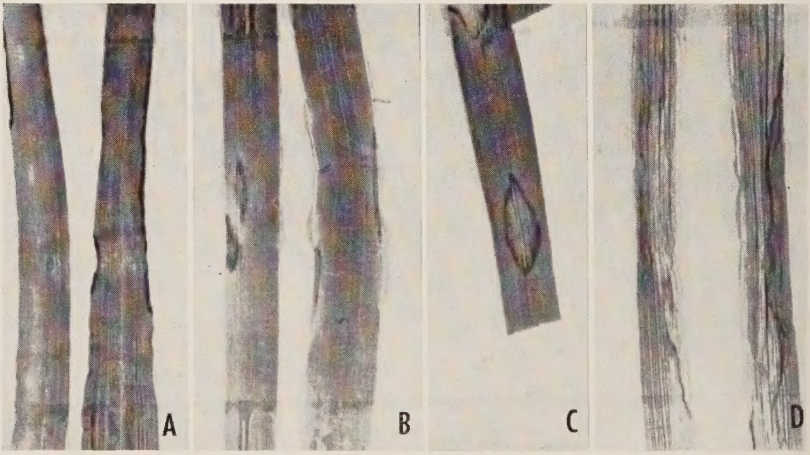


**Fig. 1. — *Rhynchosporium secalis* lesion type scale:**

**Type 0, no lesions**
**Type 1, A and B, small lesions on tips and margins of leaves**
**Type 2, C, broad lesions encompassing large areas of blade**
**Type 3, D, leaves wilted, no evidence of discrete lesions**

Final disease readings were made 16 to 19 days after inoculation. Because it was not uncommon to find 2 or 3 of the lesion types of

Sarasola and Campi on a single plant, their 5-part lesion-type scale was not employed. It was modified in the manner shown in figure 1 which combines some of the types of Sarasola and Campi with lesion descriptions by the author as follows: type 0, no lesions observed; type 1, small lesions on the tips or margins of leaves; type 2, marginal lesions extending along the blade, broad lesions encompassing a large area of the blade, or both; and type 3, leaves wilted, often with no evidence of discrete lesions *within* the wilted area.

In this connection, the work of Bryner (*1*) should be noted. He crossed susceptible and resistant plants, and inoculated the $F_2$ generation. By combining as "susceptible" all plants showing Sarasola and Campi lesion types 1 to 4, 3:1 $F_2$ ratios were obtained. He concluded that a single factor was involved, and that variation in lesion type was due to environment and handling. In the present study, plants with lesions of types 2 and 3 were called "susceptible," and a "plus" sign was used to designate this reaction.

Table 1. — Reactions of differential barley varieties of Sarasola and Campi to some United States isolates of *Rhynchosporium secalis*.

| | Isolate | | | | |
|---|---|---|---|---|---|
| Variety | Penn. | Tenn. | N. C. | Cal. 1 | Cal. 2 |
| Wisconsin Winter x Glabron | 0 | + | + | + | + |
| West China | + | + | + | + | + |
| Nigrum | + | + | + | + | + |

0 Denotes immunity or type 1 reaction.
\+ Denotes type 2 or type 3 reaction.

**RESULTS.** — *United States Races.* — Early in the study, in an effort to make this work continuous with that of Sarasola and Campi, their differential barley varieties were inoculated with five isolates of the scald disease organism from the United States. The results of these tests, summarized in table 1, indicate that some differences in specialization existed in the isolates used, but these differences were not sufficient to separate the isolates from Tennessee, North Carolina, and California. By means of these data and Sarasola and Campi's key, two races, 3 and 4, were differentiated. It can not be assumed, however, that these are identical with those in Argentina.

Further studies, in which other barleys were used, indicated that separations could be made among these five isolates and two others. Ultimately, six differential varieties were employed. Because of its usefulness in separating the Pennsylvania isolate, Wisconsin Winter x Glabron was the only variety retained of Sarasola and Campi's original list.

Table 2. — Reactions of new differential barley varieties and Wong to inoculation with eight United States isolates of *Rhynchosporium secalis*.

| VARIETY | ISOLATE | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Cal. 2 | N.C. | Mich. 1 | Cal. 1 | Tenn. | Penn. | S.D. | Mich. 2 |
| Wong (C.I. 6728) | + | + | + | + | + | + | + | + |
| Wisconsin Winter x Glabron (C.I. 8162) | + | + | + | + | + | 1 | + | + |
| Brier (C.I. 7157) | + | + | 0 | 0 | + | 0 | 0 | 0 |
| California 1311 | + | + | + | 0 | 0 | + | 0 | + |
| Hudson | + | + | 0 | 0 | 0 | 0 | 0 | 0 |
| Atlas 46 (C.I. 7323) | + | 1 | 1 | 0 | 1 | 0 | 1 | 0 |
| La Mesita (C.I. 7565) | 1 | 0 | + | 0 | 1 | 0 | 1 | 0 |

0 — no lesions.
1 — type 1 lesions.
+ — type 2 or type 3 lesions.
In some cases, plants, usually without lesions, showed a slight tip necrosis. Such plants nevertheless were called immune.

Table 2 shows the reaction of the new series of differential barleys and of the variety Wong to inoculation with eight United States isolates, and summarizes all of the studies conducted to date. 0 denotes an immune reaction; 1, resistant but not immune; and + indicates a type 2 or type 3 reaction. It was noted that Wong was susceptible to all isolates, and Wisconsin Winter x Glabron to all but the isolate from Pennsylvania. California 1311 was susceptible to 5 isolates, Brier to 3, Hudson to 2, and Atlas 46 and La Mesita to 1 each.

**Key to the races.** By use of the reactions of five of these varieties, the following key to these isolates was devised:

A a. Wisconsin Winter x Glabron resistant ..... Pennsylvania
A b. Wisconsin Winter x Glabron susceptible ..... B
B a. California 1311 resistant ..... C
C a. Brier resistant ..... California 1, South Dakota
C b. Brier susceptible ..... Tennessee
B b. California 1311 susceptible ..... C c.
C c. Brier resistant ..... D a.
D a. La Mesita resistant ..... Michigan 2
D b. La Mesita susceptible ..... Michigan 1
C d. Brier susceptible ..... D c.
D c. Atlas 46 resistant ..... North Carolina
D d. Atlas 46 susceptible ..... California 2

From table 2 and the key, it is seen that seven pathogenic types were differentiated. The two California isolates were quite different in host range. The two Michigan isolates were similar, except for the La Mesita reaction. Only the Michigan 1 isolate induced a susceptible reaction in that variety. To date, no variety has been found to differentiate the California 1 and South Dakota isolates.

That true races exist in this pathogen was demonstrated by Sarasola and Campi. The present work, with that of Houston and Ashworth (*4*) and of Reed (*8*), gives some indication of the variation of the fungus in the United States. Because contact could not be made with the Argentine authors, no cultures of their races were used in this study and nothing is known of the possible relationships of their races to these in the United States. Because of the practice of calling races by number, the following numbering system is tendered: The Sarasola and Campi races 1 to 4 could be redesignated A-1 to A-4, the "A" standing for Argentina. The United States races could then be prefixed "U.S." and numbered and determined as shown in table 3.

**Table 3. — Sources of United States barley races of *Rhynchosporium secalis* with the susceptibility of the differential barley varieties.**

| RACE | SOURCE | SUSCEPTIBLE DIFFERENTIAL BARLEYS |
|---|---|---|
| U.S. - 1 | Pennsylvania | California 1311 |
| U.S. - 2 | Tennessee | Wisconsin Winter x Glabron, Brier |
| U.S. - 3 | California<br>South Dakota | Wisconsin Winter x Glabron |
| U.S. - 4 | California | Wisconsin Winter x Glabron, Brier, California 1311, Hudson, Atlas 46 |
| U.S. - 5 | North Carolina | Wisconsin Winter x Glabron, Brier, California 1311, Hudson |
| U.S. - 6 | Michigan | Wisconsin Winter x Glabron, California 1311, La Mesita |
| U.S. - 7 | Michigan | Wisconsin Winter x Glabron, California 1311 |

## B. Susceptible Grasses

The European and Argentine work indicates that barley isolates of *Rhynchosporium* can attack non-cereal grasses. Caldwell's conclusion — that they cannot and that the grass isolates are highly specialized — stands in contradiction of this. Sprague's host range of *R. secalis* in North America (*13*) indicates that the fungus is a common, if not particularly important, grass pathogen.

As a step toward further delineating the pathogenicity of the organism, 94 species of grasses in 40 genera were inoculated with several of the isolates. Tests with the California 2 isolate have been completed. Contrary to Caldwell's conclusion, this isolate, obtained originally by Ashworth from Atlas 46, incited the scald disease on *Agropyron intermedium* (Host) Beauv., *A. pungens* (Pers.) Roem., *A. riparium* Scribn. & Smith, *A. smithii* Rydb., *A. trichophorum* (Lk.) Richt., *Bromus arvensis* L., and *Festuca idahoensis* Elmer. Re-isolations and re-inoculations of these grasses were successful.

The European culture incited lesions on *Bromus inermis* Leyss., *Elymus triticoides* Buckl., and *Agropyron smithii*, but, at the time of this writing, Koch's postulates were not fulfilled and no strong conclusions can be drawn.

## II. Resistance to the Pathogen

### A. Field Tests

A collection of 991 winter barleys from the World Collection of Small Grains was obtained in 1955 from David J. Ward, Crops Research Division, United States Department of Agriculture, Beltsville, Maryland, with the intention of screening for resistance to *Rhynchosporium secalis*. In the fall of 1955, the varieties were planted in a single nursery of 11 ranges, 100 rows per range. Rows were 3 feet long and ranges were separated by 3-foot alleys. Check rows of susceptible Wong barley were inserted after every 10 rows and spreader rows of Wong were planted around the entire nursery and in the alleys.

On the basis of their response to winter conditions in the first test, in the 1956-57 plantings the barleys were divided into three nurseries and the hardy, semi-hardy, and non-hardy types were planted in separate nurseries. These nurseries, although smaller, had check and spreader rows as already outlined. The 1955 test was conducted on the University Farm; the later test was on the Agronomy Research Farm, 14 miles east of University Park near Centre Hall. In each year, seeding was accomplished the second week in September (except, of course, the 1957 spring planting), and warm fall weather with good rainfall encouraged good stands. A strong outbreak of the barley yellow-dwarf disease in the fall of 1956 appeared to have no detrimental effect upon winter survival or reaction to the scald disease pathogen.

**Degree of coverage by natural infection.** Scald disease development in general was excellent during April and May 1956, but one area of the nursery appeared inadequately covered as evidenced by no infection or low intensity of infection of the control rows. Retesting in 1956-57 therefore was necessary. During April and May 1957, the disease was widespread in the new nurseries and none of the check rows escaped infection. Intensity was very high, some rows being almost completely defoliated.

It may be of interest that no fall symptoms of the disease were noted during either of the tests. The disease appeared in the spring, first in the border and check rows of Wong and then spread to other rows in the nurseries. The writer feels that disease occurrence was great enough in the two years to provide an adequate screening for resistance.

**General observations on the collection.** Barleys from 45 countries or locales were included in the collection. Under Pennsylvania

conditions these reacted with regard to hardiness as winter, spring, and intermediate types. The first season's test showed the collection to be divided as follows: hardy, 231 (23 per cent); semi-hardy, 524 (53 per cent); non-hardy, 236 (24 per cent). Most of the entries from India (191) and Tibet were non-hardy types as were many from Japan. Those from Korea, Transcaucasia, Turkey, and others from Japan were intermediate in their response to Pennsylvania winters. Of the 236 non-hardy types, 82 were naked barleys. No disease data were obtained from the spring plantings.

Two-row (*Hordeum distichum* L.) and six-row (*Hordeum vulgare* L.) barleys were included. A great many of those from the Near East were 2-row types. Awnleted, hooded, and smooth- and rough-awned types were included and many had anthocyanin in culms, glumes, or awns. All awnleted barleys were susceptible and only one hooded barley was resistant to scald.

**Table 4. — Countries and locales contributing barleys resistant to race U.S. - 1 of *Rhynchosporium secalis*.**

| COUNTRY | NUMBER OF ENTRIES | NUMBER RESISTANT |
|---|---|---|
| France | 20 | 12 |
| Austria | 18 | 12 |
| United States | 73 | 13 |
| Turkey | 72 | 8 |
| Germany | 31 | 5 |
| Holland | 9 | 6 |
| Belgium | 4 | 2 |
| Sweden | 6 | 1 |
| Caucasus | 25 | 1 |
| Asafute | 7 | 3 |
| Spain | 21 | 5 |
| Alaska | 2 | 2 |
| Syria | 3 | 1 |
| Other European areas | 20 | 2 |
| Total | 311 | 74 |

**Results of the tests.** After two years of testing in the field against the Pennsylvania race, only 74 varieties remained which had not shown lesions. These included 32 hardy and 42 semi-hardy barleys from 14 regions. Table 4, listing countries contributing scald-resistant barleys, indicates the total number of entries from certain areas and the number of these which were resistant to the Pennsylvania race.

Tables 5 and 6 contain the names, Cereal Investigation numbers, species, heights, head characteristics, and sources of the 74 barleys resistant to the Pennsylvania race in field tests.

Table 5. — Winter hardy barleys resistant to race U.S. - 1 of *Rhynchosporium secalis*.

| NAME | CI | 6-ROW | 2-ROW | HEIGHT INCHES | HEAD TYPE | SOURCE |
|---|---|---|---|---|---|---|
| LaFite | 8289* | X | | 24† | A-R‡ | France |
| Gembloux 006 | 8290* | X | | | A-R | " |
| Groninger | 8292* | X | | 30 | A-R | " |
| | 8981 | | X | 21 | A-S | Syria |
| | 9043* | X | | 30 | A-R | Austria |
| | 9054 | X | | 24 | A-R | " |
| | 9250 | X | | 24 | A-R | France |
| Ondine | 9252* | X | | 24 | A-R | " |
| Admonter-3 | 9889* | X | | 24 | A-S | Austria |
| Tenn. Winter (Sel.) | 876* | X | | 26 | A-R | Europe |
| Cusada | 895* | X | | 26 | A-R (red) | Md.-Hyb. |
| Arabel | 896* | X | | 28 | A-R | USDA-Hyb. |
| | 2420 | X | | 33 | A-R | Calif. |
| Guilder | 2485* | X | | 28 | A-S | Holland |
| Old Ambster Winter | 2491* | X | | 30 | A-S | " |
| Kroon | 2495* | X | | 30 | A-R | " |
| | 2544* | X | | | A-R | Italy |
| Polders | 3213 | X | | 26 | A-R | Belgium |
| | 3346* | X | | 30 | A-R (red) | Caucasia |
| Alaska | 4106 | X | | 32 | A-R | Alaska |
| | 4678* | X | | 30 | A-R (red) | Kentucky |
| Dutch Winter | 5950* | X | | 22 | A-R | Holland |
| | 6685* | X | | 26 | A-R | Asafute |
| Fimbul | 7321* | X | | 26 | A-R | Sweden |
| Tschermak | 7508* | X | X | 26 | A-R | Germany |
| | 7524* | X | | 26 | A-R | Okla.-Hyb. |
| | 7571* | X | | 27 | A-R | Mo.-Hyb. |
| | 8067* | X | | 27 | A-R | N.Y.-Hyb. |
| Pueblo | 8070* | X | | 27 | A-R | Colorado |
| | 8795* | X | | 28 | A-R | Germany |
| | 9046* | X | | 26 | A-R | Austria |
| | 9050* | | X | 26 | A-R | " |

* Tested later against California race U.S. - 4.
† Height probably reduced by barley yellow-dwarf virus.
‡ A — awned, R — rough, S — semi-rough.

Besides the 191 from India, other large sources of entries were Japan, Korea, China, the United States, Transcaucasia, and Turkey. Table 7 indicates the number of entries from these sources and the numbers of these that were resistant to race U.S. 1.

### B. Results with Other Pathogen Races

**Initial greenhouse screening.** Fifty-two of the 74 scald-resistant, winter hardy, and semi-winter hardy varieties, indicated with asterisks in tables 5 and 6, were tested against *Rhynchosporium* race U.S. - 4

Table 6. — Semi-winter-hardy barleys resistant to race U.S. - 1 of *Rhynchosporium secalis.*

| NAME | CI | 6-ROW | 2-ROW | HEIGHT INCHES | HEAD TYPE | SOURCE |
|---|---|---|---|---|---|---|
| | 8116 | X | | 15† | A-R‡ | Turkey |
| | 8119* | X | | 25 | A-R (red) | " |
| | 8251* | X | | 16 | A-S | " |
| | 8256 | X | | 15 | A-S | " |
| Mammouth - 1 | 8281* | X | | 28 | A-R | France |
| Mammouth - 2 | 8282* | X | | 26 | A-R | " |
| Gembloux - 14 | 8286* | X | | 24 | A-R | " |
| Baillargi | 8324 | X | | | | " |
| | 8618* | | X | 18 | A-R | Turkey |
| | 8624* | X | | 22 | A-R | " |
| | 8725* | | X | | A-S | " |
| | 8726* | | X | | A-S | " |
| | 8790* | | X | 24 | A-S | Germany |
| | 8977* | X | | 22 | A-R | Syria |
| | 9042* | X | | 28 | A-S | Austria |
| | 9051* | | X | 24 | A-S | " |
| Probstdorf | 9254* | | X | 26 | A-R | " |
| Lasser | 9893* | X | | 28 | A-S | " |
| Vindicat | 9948* | X | | 26 | A-R | Holland |
| Alaska | 534* | X | | 30 | A-S | Alaska |
| | 3367-2* | | | | | Algeria |
| | 3515* | | | 24 | A-R | Spain |
| | 3516 | | | 26 | A-S | " |
| | 3522 | X | | 26 | A-S | Spain |
| | 4677* | X | | 32 | A-R | Kentucky |
| | 5561* | X | | 30 | A-S (red) | Asafute |
| | 5562* | X | | 31 | A-S | " |
| Olympia | 6107* | X | | 24 | A-R | Germany |
| Davidson | 6373* | X | | 28 | A-S | N.C.-Hyb. |
| Grossklopige | 6485* | X | | 24 | A-S | Germany |
| NC - 11 | 6564* | X | | 28 | A-SR | N.C.-Hyb. |
| | 7076 | X | | 30 | Hooded | Tenn. |
| Lighee - 14 | 7283 | X | | 24 | A-S | Belgium |
| Bayyax | 7484 | X | | 24 | A-S | Spain |
| Vima | 7528 | X | | 26 | A-R | Holland |
| | 7585 | | X | 24 | A-S | Austria |
| | 7586 | X | | 24 | A-S | " |
| Harriet | 7590 | X | | 30 | A-S | " |
| | 8069 | X | | 30 | A-S | N.Y.-Hyb. |
| Albert | 8271 | X | | | A-S | France |
| Wagonville | 8273 | X | | | A-R | " |
| Wieland | 8277 | X | | 27 | A-S | " |

* Tested later against California race U.S. - 4.
† Height probably reduced by barley yellow-dwarf virus.
‡ A — awned, R — rough, S — semi-rough.

**Table 7. — Geographical distribution of race U.S. - 1 resistance to *Rhynchosporium secalis*.**

| SOURCE | NUMBER OF ENTRIES | NUMBER RESISTANT |
|---|---|---|
| Japan | 149 | 0 |
| Korea | 96 | 0 |
| India | 191 | —* |
| China | 86 | 0 |
| United States | 73 | 13 |
| Turkey | 72 | 8 |
| Transcaucasia | 52 | 0 |

* Winter-killed.

**Table 8. — Responses of winter hardy barleys to inoculation with race U.S. - 4 of *Rhynchosporium secalis*.**

| VARIETY OR CI NUMBER | NUMBER OF PLANTS INFECTED | TOTAL PLANTS IN TEST | LESION TYPE |
|---|---|---|---|
| Tenn. Winter (876) | 8 | 8 | 2 |
| 6685 | 8 | 10 | 2 |
| 8981 | 7 | 7 | 2 |
| Tschermak (7508) | 6 | 6 | 2 |
| 4678 | 11 | 11 | 2 |
| Arabel (896) | 7 | 8 | 2 |
| Admonter - 3 (9889) | 10 | 10 | 2 |
| 9046 | 10 | 10 | 2 |
| 7571 | 7 | 19 | 2 |
| Polders (3213) | 10 | 11 | 2 |
| 9050 | 11 | 13 | 2 |
| 9043 | 5 | 7 | 2 |
| 7524 | 9 | 12 | 2 |
| Alaska (4106) | 11 | 12 | 2 |
| Pueblo (8070) | 6 | 15 | 2 |
| 2420 | 9 | 11 | 2 |
| Old Ambster (2491) | 6 | 12 | 2* |
| 3346 | 7 | 11 | 2 |
| 9250 | 6 | 6 | 2 |
| 9054 | 8 | 8 | 2 |
| Ondine (9252) | 7 | 7 | 2 |
| Dutch Winter (5950) | 10 | 11 | 2 |
| Cusada (895) | 13 | 13 | 2 |
| LaFite (8289) | 6 | 6 | 2* |
| 8795 | 8 | 12 | 2 |
| 2544 | 6 | 7 | 2 |
| Groninger (8292) | 7 | 7 | 2 |
| Kroon (2495) | 11 | 12 | 2* |
| Guilder (2485) | 6 | 8 | 2 |

* Later used in all-races test.

Table 9. — Responses of semi-winter hardy barleys to inoculation with race U.S. - 4 of *Rhynchosporium secalis*.

| VARIETY OR CI NUMBER | NUMBER OF PLANTS INFECTED | TOTAL PLANTS IN TEST | LESION TYPE |
|---|---|---|---|
| 8790 | 3 | 10 | 2 |
| Bailargi (8324) | 6 | 9 | 2 |
| Probstdorf (9254) | 4 | 15 | 2 |
| 8251 | 0 | 12 | * |
| 5561 | 13 | 13 | 2 |
| 3622 | 0 | 8 | * |
| Fimbul | 3 | 11 | 2 |
| 5562 | 11 | 14 | 2 |
| 8067 | 4 | 14 | 2 |
| 7677 | 7 | 11 | 2 |
| 8119 | 12 | 13 | 2 |
| 8977 | 9 | 11 | 2 |
| Wagonville (8273) | 6 | 9 | 2 |
| 8069 | 9 | 9 | 2 |
| Bayyax (7484) | 7 | 11 | 2 |
| Alaska (534) | 10 | 12 | 2 |
| Harriet (7590) | 10 | 10 | 2 |
| Grossklopige (6485) | 8 | 10 | 2 |
| Olympia (6107) | 7 | 7 | 2 |
| Gembloux - 14 (8286) | 0 | 10 | * |
| Lasser (9893) | 7 | 10 | 2 |
| 3516 | 10 | 15 | 2 |
| 8725 | 13 | 16 | 2 |
| 8624 | 0 | 6 | * |
| NC - 11 | 8 | 8 | 3 |
| 3515 | 3 | 14 | 1* |
| Wieland (8277) | 8 | 8 | 2 |
| 7585 | 8 | 9 | 2 |
| 8116 | 3 | 11 | 2 |
| Mammouth - 2 (8282) | 10 | 10 | 2 |
| Mammouth - 1 (8281) | 3 | 15 | 2 |
| 9042 | 7 | 8 | 2 |
| 8618 | 0 | 14 | * |
| Lignée - 14 (7283) | 0 | 13 | * |
| 8726 | 14 | 15 | 2 |
| Albert (8271) | 2 | 11 | 1* |
| 8256 | 0 | 3 | * |
| 7076 | 8 | 10 | 2 |
| Davidson (6373) | 11 | 11 | 2 |
| Vindicat (9948 or 6596) | 1 | 10 | 2* |
| 7586 | 8 | 8 | 2 |
| 3523 - 1 | 8 | 9 | 2 |
| Vima (7528) | 0 | 9 | * |

* Later used in all-races test.

from California, a pathogen with a wider host range than U.S. - 1. None of the 26 winter hardy varieties tested against this race in the greenhouse were resistant; but three, because of superior agronomic performance, were tested later against all races. Of the 26 semi-hardy varieties tested, 11 showed sufficient resistance to be included in the all-races test. Tables 8 and 9 contain the data yielded by these tests.

**The all-races test.** The afore-mentioned 11 semi-hardy and the three hardy barleys together with Wong as a susceptible control were inoculated in the greenhouse, techniques described previously being used. Results of this test are shown in table 10. If the response of Wong as a control variety is used as a measure of the intensity of the test, it can be concluded that the 14 lines were subjected to very severe disease conditions.

Three varieties were immune to the seven races inasmuch as no lesions were observed. These were CI 3515 (Spain); CI 8256 (Turkey); and Gembloux - 14, CI 8286 (France). In addition, CI 8618 (Turkey) can be classed as resistant, if not immune, having shown only small lesions in response to races U.S. - 1 and U.S. - 3 and auricle lesions in response to the new North Carolina isolate. This last isolate, called U.S. - 5 in the table, was actually never tested against the differential varieties to determine if it was identical to the North Carolina isolate used in the previous race determination studies but lost from culture.

Three varieties emerged from the test resistant to all but 1 race. Of 14 plants of CI 8251 (Turkey) inoculated, 2 showed susceptible-type lesions in response to race U.S. - 1, and 1 plant of 7 inoculated showed a resistant-type reaction in response to race U.S. - 6. Vima (CI 7528) was susceptible to race U.S. - 4 only, and immune to all others. Lignee - 14 (CI 7283) was susceptible to race U.S. - 6 and immune to all others.

CI 3522 was susceptible to races U.S. - 1 and U.S. - 7, gave resistant responses to races U.S. - 4 and U.S. - 5, and was immune to the other three races. The three winter hardy varieties in the test, Lafite, Old Ambster, and Kroon, performed rather poorly. Lafite showed immunity to three races, U.S. - 2, U.S. - 6 and U.S. - 7, but Old Ambster and Kroon were susceptible to all races.

## Discussion

That pathogenic specialization exists in *Rhynchosporium secalis* is not surprising, and to find it among isolates collected from different barley growing areas of the United States was to be expected. One may ponder in what way an asexual organism becomes so specialized, but the work of fungus geneticists and physiologists has shown that through mutation and heterokaryosis these organisms may become

**Table 10. — Responses of 14 barleys from the world collection and Wong to inoculation with all isolated U.S. races of *Rhynchosporium secalis*.**

| VARIETY OR CI NUMBER | U.S. - 1 (Penn.) | | U.S. - 2 (Tenn.) | | U.S. - 3 (Cal.) | | U.S. - 4 (Cal.) | | U.S. - 5° (N.C.) | | U.S. - 6 (Mich.) | | U.S. - 7 (Mich.) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ext.† | L.T.‡ | Ext. | L.T. | Ext. | L.T. | Ext. | L.T. | Ext. | L.T. | Ext. | L.T. | Ext. | L.T. |
| 3515 | 0/10 | | 0/10 | | 0/16 | | 0/13 | | 0/9 | | 0/11 | | 0/9 | |
| 8256 | 0/12 | | 0/10 | | 0/8 | | 0/13 | | 0/15 | | 0/9 | | 0/7 | |
| Gembloux - 14 (8286) | 0/14 | | 0/12 | | 0/12 | | 0/16 | | 0/14 | | 0/11 | | 0/12 | |
| 8618 | 2/11 | 1 | 0/16 | | 8/9 | 1 | 0/10 | | 9/9 aur. | | 1/13 | | 0/13 | |
| 8251 | 2/14 | 2 | 0/8 | | 0/9 | | 0/11 | | 0/10 | | 1/7 | 1 | 0/11 | |
| Vima (7528) | 0/9 | | 0/12 | | 0/11 | | 15/16 | 2 | 0/12 | | 0/10 | | 0/8 | |
| Lignee - 14 (7283) | 0/9 | | 0/10 | | 0/9 | | 0/13 | | 0/9 | | 9/12 | 2 | 0/11 | |
| 8624 | 1/6 | 3 | 0/4 | | 0/3 | | 1/5 | 2 | 0/6 | | 6/6 | 2 | 2/7 | 3 |
| 3522 | 8/8 | 2, 3 | 0/10 | | 0/9 | | 5/5 | 1 | 8/8 | 1 | 0/8 | | 9/9 | 2 |
| Albert (8271) | 17/20 | 1, 2 | 1/16 | 2 | 13/13 | 1, 2 | 9/9 | 2, 3 | 3/11 | 1 | 0/14 | | 0/13 | |
| LaFite (8289) | 5/12 | 1 | 0/9 | | 6/10 | 1-3 | 14/14 | 2 | 1/9 | 2 | 0/8 | | 0/11 | |
| Old Ambster (2491) | 12/12 | 3 | 5/7 | 2 | 14/14 | 3 | 8/8 | 2 | 15/15 | 2 | 10/10 | 2 | 8/10 | 2 |
| Kroon (2495) | 12/12 | 3 | 11/13 | 2 | 9/9 | 3 | 11/12 | 2 | 8/12 | 2 | 9/9 | 2 | 12/13 | 2 |
| Vindicat (9948) | 14/14 | 2 | 9/13 | 2 | 13/14 | 3 | 9/10 | 1, 2 | 11/11 | 2 | 9/9 | 2 | 22/22 | 2 |
| Wong (6728) | 13/13 | 3 | 13/13 | 2, 3 | 14/14 | 3 | 13/13 | 2, 3 | 19/19 | 3 | 13/13 | 3 | 14/14 | 3 |

° This was a new isolate from North Carolina not yet tested or known to be identical with original U.S. - 5.

† Extensity is the number infected plants over the total in the test.

‡ L.T. designates the lesion type as indicated earlier.

quite varied. Because *Rhynchosporium* is uninucleate, heterokaryotic phenomena probably do not apply, but there still is the possibility of mutation, of adaptation, or of an undiscovered sexual stage.

Probably one could go on screening new isolates against the differential varieties and turn up dozens of pathogenic types. This was not and is not now our aim. It is hoped that this work demonstrates adequately that pathogenic specialization exists and that the few lines derived from the hundreds screened will have real use in giving broad-spectrum scald-disease resistance to American barleys. It is interesting to note that no broad resistance was found in the winter-hardy lines tested. The question also arises of whether there is significance in the number of resistant lines which originated in the Middle East. Barley is native to this area, and through centuries of culture strains susceptible to *Rhynchosporium secalis* and other diseases may have disappeared. Intensive screening of germplasm from there might be fruitful from the standpoint of disease resistance.

## III. Cultural Variability

### A. Responses to Different Media

When work was started on this project, it was difficult to obtain sufficient growth and sporulation of *Rhynchosporium* to keep a sufficient supply of inoculum on hand for pathogenicity tests. Fourteen different media were made up according to the recipes listed which are based on liter quantities. Flaked agar was added to each in the amount of 20 grams per liter with the exceptions of commercially prepared Lima bean and malt extract agars (Difco).

(a) Potato sucrose agar (PS): 200 g diced potatoes boiled in 500 ml $H_2O$; filtered through cheesecloth; filtrate only used; 2 g sucrose added.

(b) Cornmeal agar (C): 10 g cornmeal boiled in 500 ml $H_2O$ for 5 minutes; filtered through cheesecloth; filtrate only used.

(c) Oatmeal agar (O): 10 g oatmeal boiled in 500 ml $H_2O$ for 5 minutes; filtered through cheesecloth; filtrate only used.

(d) Cornmeal-oatmeal agar (CO): combine 10 g each. Follow preceding directions.

(e) Lima bean agar (LB): 22 g 'Difco' dehydrate; 5 g agar added since this preparation already contained 15 g agar per liter.

(f) Malt extract agar (ME): 10 g 'Difco' malt extract.

(g) Lima bean-malt extract agar (LM): combination of two previous, e and f, preparations.

(h) Barley grain infusion agar (BG): 20 g barley grain crushed and boiled in 500 ml $H_2O$ for 5 minutes; filtered through cheesecloth; filtrate only used.

(i) Yeast extract agar (YE): 10 g 'Difco' yeast extract.

(j) V-8 juice agar (V-8): 100 ml commercial V-8 juice; 1.5 g $CaCO_3$ added to reduce acidity.

(k) Nutramigen agar (N): 17 g Nutramigen; Nutramigen is a product of the Mead-Johnson Company.

(l) Rice polish agar (P): 10 g rice polish boiled in 500 ml $H_2O$ for 5 minutes; filtered through cheesecloth; filtrate only used.

(m) Wheat bran infusion agar (WB): 10 g wheat bran boiled in 500 ml $H_2O$ for 5 minutes; filtered through cheesecloth; filtrate only used.

(n) Water agar (WA): 20 g agar melted in 1 liter water.

Three 50 ml Erlenmeyer flasks were filled to their greatest diameter with each of these media and 10 ml of each were added to each of 3 petri dishes. Each plate and flask of medium was inoculated with a single drop of conidial spore suspension which had been adjusted through use of a haemocytometer and dilution so that each flask received approximately 400 spores and each plate 5000 spores. Plates and flasks were then placed in a 20° C chamber and received approximately 300 footcandles of fluorescent light during alternating 12-hour periods. Colony diameters were measured after 10, 16, and 22 days growth in the plates. The flask cultures were used for a spore production assay on the sixteenth day of growth. To each was added 10 g of 3 mm glass beads and 5 ml of water and each complete replicate (all media) was shaken for 5 minutes on a Burrel shaker and spore counts were made with a haemocytometer. The Pennsylvania isolate of *Rhynchosporium* was used.

Table 11, "Responses of the Pennsylvania isolate of *Rhynchosporium secalis* to various media" summarizes the data of these experiments. It should be necessary here but to point out a few significant points. Greatest growth occurred on rice-polish medium and was almost equalled by that on potato-sucrose agar. Best sporulation was on Lima bean agar on which growth was very poor. These colonies were, however, almost entirely spores. Possibly the best medium for general work is potato-sucrose agar on which is obtained good growth and also good sporulation. None of these media solved the laboratory problem of slow growth inasmuch as on the best medium the colony attained a diameter of only 22 millimeters in 22 days. The experiment was of practical use in that Lima bean agar was used in all later studies for inoculum production and very large amounts of spores were always available for use.

### B. Responses to pH

Three studies were carried out to explore the effect of pH on *Rhynchosporium*, effect of pH on spore germination at 18° C, effect

**Table 11. — Responses of the Pennsylvania isolate of *Rhynchosporium secalis* to various media.**

| Medium | Colony Character | Colony Diameters at Various Ages | | | Spores per ml |
|---|---|---|---|---|---|
| | | 10 days | 16 days | 22 days | |
| | | *Mm* | *Mm* | *Mm* | *Millions* |
| Water agar | Sparse white aerial growth; slight subsurface dendriform growth | 5.8 | 5.9 | 6.1 | 0 |
| Lima bean agar | Pink, yeast-like surface growth | 8.4 | 9.4 | 11.3 | 15.5 |
| Yeast extract agar | Pink, yeast-like border on central raised, furrowed stroma; no subsurface growth | 9.0 | 10.0 | 12.6 | .67 |
| Lima-malt extract agar | Dark brown, furrowed, erumpent stroma | 4.5 | 8.8 | 14.5 | 3.9 |
| V-8 juice agar | Pink to light brown, radially furrowed stroma with hyphal fringe | 9.2 | 11.0 | 15.0 | 2.6 |
| Malts extract agar | Dark-brown stroma with considerable subsurface mycelium | 7.4 | 9.4 | 15.2 | 3.1 |
| Barley grain decoction agar | Sparse, cream-colored surface growth with dendriform subsurface growth | 9.0 | 10.8 | 15.2 | .69 |
| Nutramigen agar | Pink, convoluted, furrowed stroma | 9.0 | 11.0 | 16.0 | .06 |
| Cornmeal agar | Tan, dendriform surface growth; heavy radial strands; some subsurface growth | 9.0 | 10.8 | 17.0 | .71 |
| Wheat bran agar | Sparse, white, dendriform surface growth | 8.5 | 12.5 | 17.3 | 2.13 |
| Oatmeal agar | Dark-brown, stromatic surface growth | 9.6 | 12.2 | 17.8 | .5 |
| Cornmeal-oatmeal agar | Dark-brown, mostly subsurface growth | 11.2 | 13.0 | 19.0 | .5 |
| Potato-sucrose agar | Brown, radially furrowed, raised colony; hyphal fringe | 9.0 | 13.5 | 21.0 | 8.9 |
| Rice polish agar | Dark-brown stroma with deep penetration into agar | 10.0 | 12.6 | 22.0 | 1.00 |

on spore germination in the temperature range 3 to 30° C, and effect on radial growth of the fungus in petri dishes at 18° C. The isolates of the fungus employed, designated I, II, and III, originated in Pennsylvania, California, and Europe, respectively.

For the study of the effect of pH on growth, 1 per cent malt extract in 2 per cent agar was adjusted in pH by pipetting N/2 HCl or NaOH to the petri dish before pouring. Before the medium solidified, pH values were checked with an electronic pH meter. After solidification, the agar was inoculated with a 1 mm loopful of a suspension of $10^5$ spores/ml, isolate I being used in this test. The loop was touched once in the plate center. Three plates at each of the following pH's: 2.0, 2.5, 3.0, 4.0, 5.3, 6.5, 8.0, 9.5 and 10.5, were prepared. After 2 weeks' incubation at 18° C, the colony diameters were measured in millimeters. The results, depicted graphically in figure 2, show that growth at pH 6.5 was optimum followed by 8.0, 5.3 and 9.5. Growth was nil at pH 2.0, 2.5 and 10.5.

Several spore germination tests were conducted in which all three isolates were used. In all, conidia were from 10-day old transfer cultures on Lima bean agar. Suspensions of spores were made, adjusted to 25,000 spores/ml, divided into aliquots, and each aliquot adjusted to a particular pH value with 0.1N HCl and NaOH. The pH values used were 4.0, 6.0, 7.0, 8.0 and 10.0. Germinations were conducted on glass slides on which 3 small circles were inscribed with a wax pencil. A drop of spore suspension from a pipette was placed in each of these circles and 3 slides were used at each pH value at each temperature. One slide was read after 12 hours, another after 24 hours, and the third after 36 hours. The temperatures of incubation were

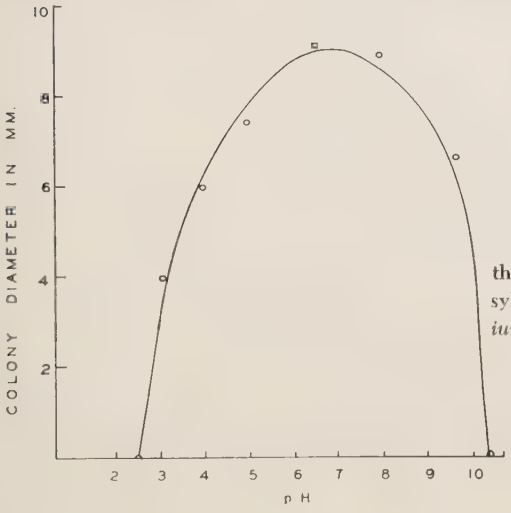


**Fig. 2. — Effect of pH on the colony diameter of Pennsylvania isolate of *Rhynchosporium secalis*.**

3, 6, 9, 12, 15, 18, 21, 24, 27, and 30° C. During incubation, the slides were kept in closed petri dishes to which small amounts of water had been added to provide moisture-saturated atmospheres.

The three isolates responded in almost identical manner to the test conditions. Figure 3 depicts graphically the results of tests with isolate I. Best early germination occurred at 18° C at pH 4.0 and 6.0, fig. 3A. After 24 hours, spores at pH 4, 6, and 8 had attained 100 per cent germination at 18° C, fig. 3B. The temperature response curve is markedly peaked at all pH values except 7.0 in which only a low level of germination occurred. After 36 hours, 100 per cent germination was achieved at both 18° and 20° C by spores at pH values of 4, 6, and 8, fig. 3C. The greater response at the lower and higher temperatures by spores at pH 8 is striking. Spores at pH 7 still had not reached a high level of germination.

No explanation could be made of the low response at pH 7 but a trial was made at 18° C, in which buffered solutions of 0.1 M citric acid and 0.2 M potassium mono-hydrogen phosphate were used. In this test, a germination percentage of almost 100 was reached in 24 hours at pH 7, but a considerable depression of the curve occurred at pH 7.6. This line of investigation was not pursued further.

### C. Responses to Various Vitamins and Carbon and Nitrogen Sources

A single, replicated experiment in which all three isolates were used was conducted to determine the response of the fungus to some sources of nitrogen and carbon and to some vitamins. To accomplish this, 'Difco' yeast base media of 3 types were used, namely, B 391, B 392, and B 394 (5). Each of these media contains salts providing the major and minor nutritional elements. The nitrogen base medium contains 5 g/l ammonium sulfate plus 3 amino acids and 9 vitamins but lacks a carbon source. The carbon base medium lacks ammonium sulfate, has one-tenth the amino acid concentration of the nitrogen base medium, and contains 10 g/l of dextrose and 9 vitamins. The vitamin-free medium has ammonium sulfate, dextrose, and full concentration of amino acids, but has no vitamins.

To the nitrogen base medium were added the following carbon sources in a concentration of 1 per cent by weight: sucrose, glucose, sorbose, ethyl alcohol, wheat starch, and maltose. Each carbon source was autoclaved separately and added to the basal medium aseptically through the technique of McKeen (6). The base medium pH was 6.7.

To the carbon base medium were added the following nitrogen sources in amounts to equal that amount of nitrogen in 5 g of ammonium sulfate: asparagine, urea, and potassium nitrate. The final pH was 7.0.

To the vitamin-free medium were added the following in the concentrations indicated: thiamine (400 ug/l), biotin (2 ug/l), thiamine

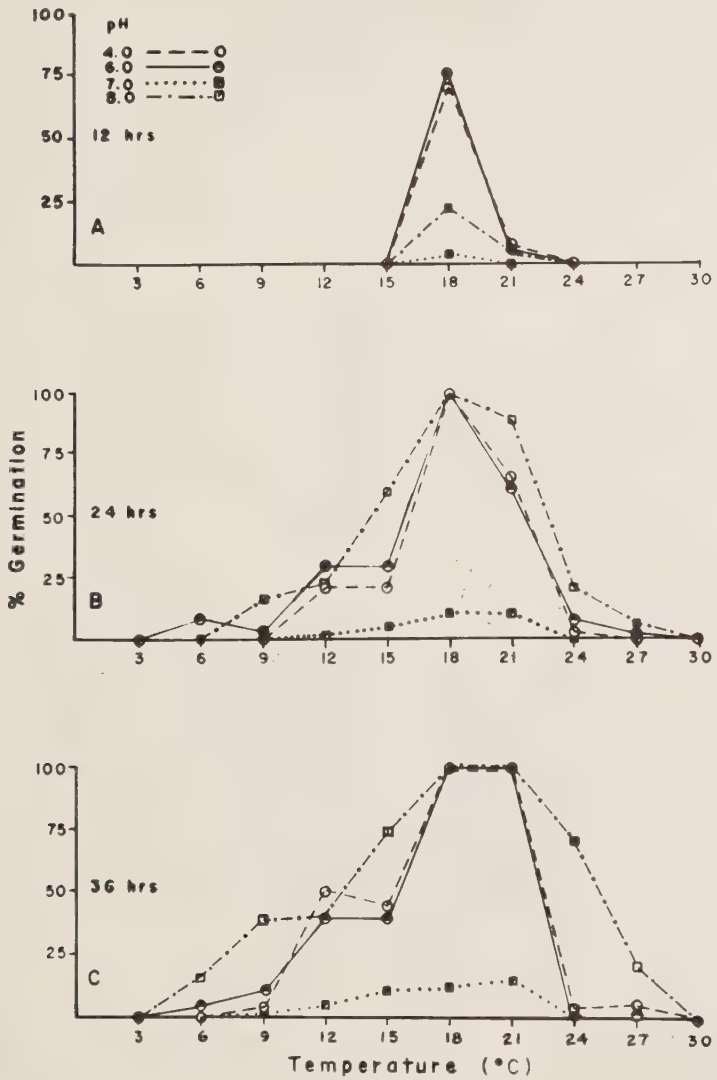


**Fig. 3. — Effect of pH and temperature on the percentage germination of the Pennsylvania isolate of *Rhynchosporium secalis*.**

(400 ug/l) plus biotin (2 ug/l), and yeast extract (0.1 g/l). The final pH was 6.7.

To each medium was added 15 g/l of flaked agar, and the completed media were poured into sterile petri dishes. Three replications

per variable were prepared. One, 1 mm loopful of a suspension of 100,000 spores/ml was placed in the center of each dish, and the cul-

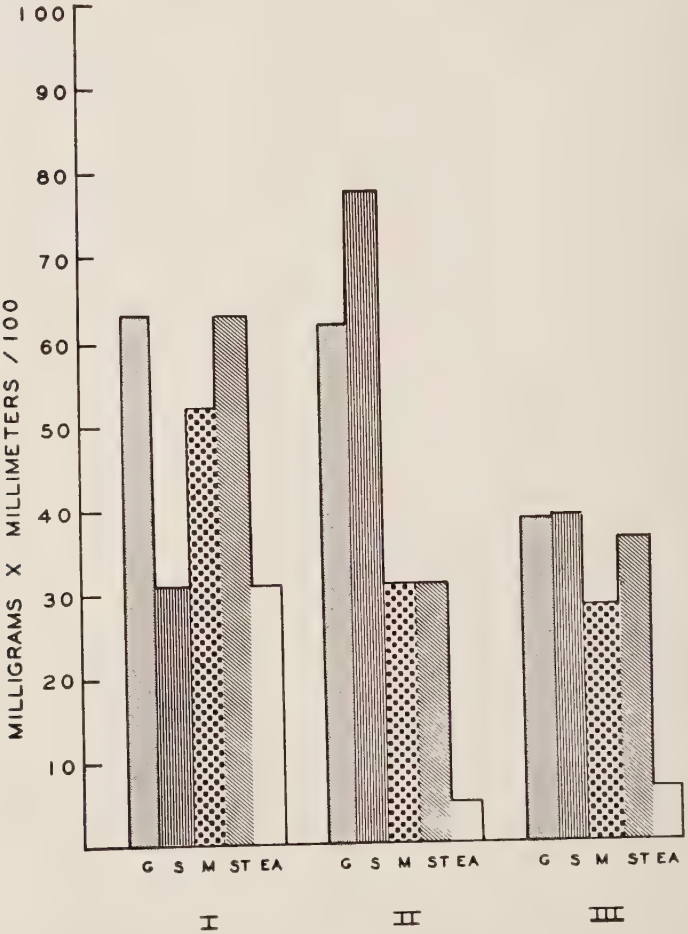


**Fig. 4. — Responses of three isolates of *Rhynchosporium secalis* to five carbon sources.**

| Carbon Source | Isolate I | | | Isolate II | | | Isolate III | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | *Mg X Mm* | | | *Mg X Mm* | | | *Mg X Mm* |
| | *Mg* | *Mm* | *100* | *Mg* | *Mm* | *100* | *Mg* | *Mm* | *100* |
| Glucose | 370 | 17 | 63 | 440 | 14 | 62 | 320 | 12 | 38 |
| Sucrose | 260 | 12 | 31 | 460 | 17 | 78 | 325 | 12 | 39 |
| Maltose | 260 | 20 | 52 | 340 | 9 | 31 | 280 | 10 | 28 |
| Starch | 300 | 21 | 63 | 310 | 10 | 31 | 240 | 15 | 36 |
| Ethyl alcohol | 180 | 12 | 31 | 120 | 4 | 5 | 110 | 6 | 7 |

tures were incubated at 18° C for 3 weeks. Growth was recorded as colony diameter and dry weight of mycelium.

**RESULTS. Responses to different carbon sources.** Figure 4 and associated tabulation present the results of this experiment. Measurements of colony diameters in millimeters and milligrams of mycelium

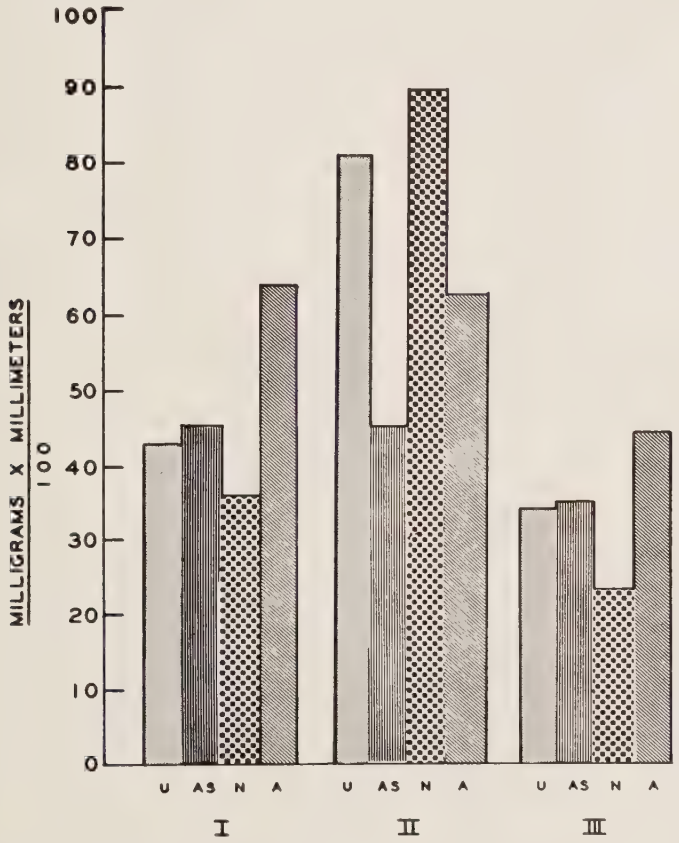


**Fig. 5. — Responses of three isolates of *Rhynchosporium secalis* to four nitrogen sources.**

| Nitrogen Source | Isolate I | | | Isolate II | | | Isolate III | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | *Mg X Mm* | | | *Mg X Mm* | | | *Mg X Mm* |
| | *Mg* | *Mm* | *100* | *Mg* | *Mm* | *100* | *Mg* | *Mm* | *100* |
| Urea (u) | 250 | 17 | 42.5 | 440 | 18 | 80.2 | 285 | 12 | 32.2 |
| Asparagine (a) | 320 | 14 | 44.8 | 410 | 11 | 45.1 | 295 | 12 | 35.4 |
| $KNO_3$ (N) | 300 | 12 | 36.0 | 500 | 14 | 90.0 | 235 | 10 | 23.5 |
| $NH_4NO_3$ (A) | 370 | 17 | 62.9 | 445 | 14 | 62.3 | 325 | 12 | 39.0 |

produced are given. Because of difficulty in appraisal of these data, each two measurements were multiplied together, and the resulting figures divided by 100 are presented in the graph. Without rendering absolutely quantitative judgment, it can be said that (1) the isolates

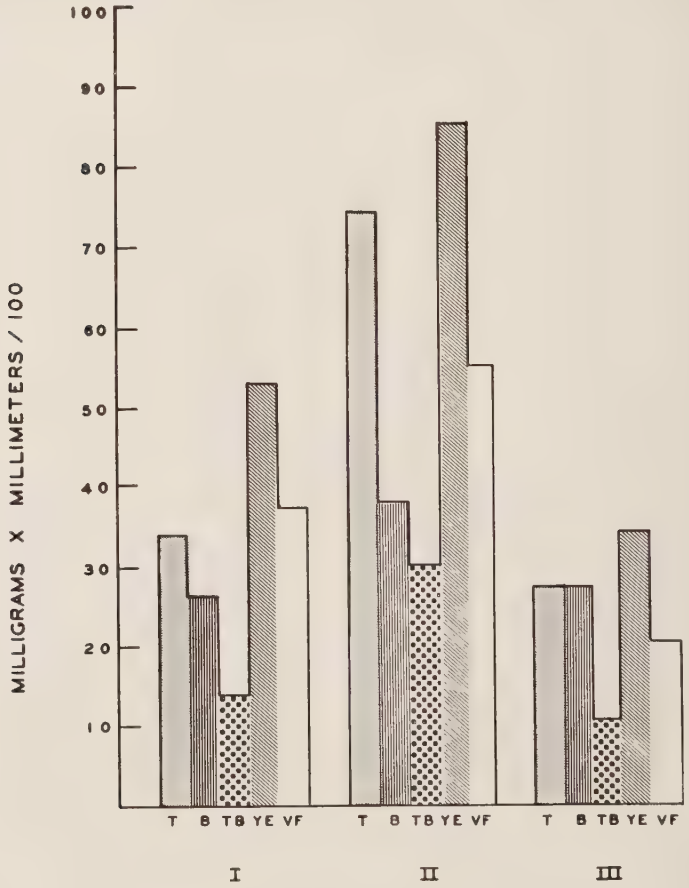


**Fig. 6. — Responses of three isolates of *Rhynchosporium secalis* to various vitamin sources.**

| Vitamin Source | Isolate I | | | Isolate II | | | Isolate III | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | *Mg X Mm* | | | *Mg X Mm* | | | *Mg X Mm* |
| | *Mg* | *Mm* | *100* | *Mg* | *Mm* | *100* | *Mg* | *Mm* | *100* |
| Thiamine (T) | 275 | 12 | 33 | 540 | 14 | 75 | 210 | 13 | 27.3 |
| Biotin (B) | 180 | 15 | 27 | 300 | 13 | 39 | 195 | 14 | 27.3 |
| T + B | 175 | 8 | 14 | 300 | 10 | 30 | 150 | 7 | 10.5 |
| Yeast extract (TE) | 260 | 20 | 52 | 425 | 20 | 85 | 195 | 16 | 31.2 |
| Vitamin-free | 220 | 18 | 39 | 325 | 17 | 55.2 | 190 | 10 | 19.0 |

varied in their utilization of the substrates, (2) isolates I and II were able to utilize glucose better than isolate III, (3) isolate II was markedly more able to use sucrose, and (4) isolate I was most capable of using maltose, starch, and ethyl alcohol; isolates II and III were about equal in ability to utilize these three substrates.

**Responses to different nitrogen sources.** Figure 5 and associated tabulation present the results of this experiment. Data were handled as in the previous study. They indicate (1) that isolate II equalled or exceeded the abilities of the other isolates to utilize all nitrogen sources in the study, (2) that isolate I was, in turn, better able to use all sources than isolate III and, (3) that no sharp distinctions can be made as to the ability of the organism to utilize nitrogen from inorganic or organic sources.

**Responses to various vitamin sources.** Figure 6 and its tabulation present the results of this study in the same manner as in the previous two experiments. The same pattern of use of the vitamins was followed by all three isolates but isolate II gave much greater responses. In addition, thiamine plus biotin always allowed less growth than either thiamin or biotin alone or the vitamin-free medium. The uncharacterized yeast extract always gave the greatest effect. The response to thiamine was great only with isolate II, and biotin always allowed less growth than the vitamin-free medium.

### D. Enzyme studies with Rhynchosporium

When *Rhynchosporium secalis* is grown on laboratory media in petri dishes, growth proceeds very slowly and stops after about three weeks. As can be seen from responses already discussed, the diameter of a colony seldom exceeds 20 mm. The reasons for this determinate growth were explored when it was noted, quite by accident, that a large clear ring surrounded the fungus on starch media when the plates were flooded with Lugol's iodine reagent, fig. 7. In recognition of this indication that the starch of the medium had been hydrolized well in advance of the fungus, a series of studies of the three isolates was conducted to learn more about this enzymatic activity.

First, efforts were directed at proving that the organism possessed starch hydrolyzing activity. In testing each isolate a series of petri dishes of Nutramigen agar was inoculated with a 1 mm loopful of a suspension of 100,000 spores/ml. After 8 days growth at 18° C, a sample plate was flooded with Lugol's reagent. The diameter of the cleared zone, about 2 cm, was noted and areas of similar size were cut from 20 other cultures. The mycelium was excluded. This agar was mashed with a mortar and pestle and allowed to stand 24 hours in 100 ml of 0.1 M NaCl. This mixture was filtered to remove the agar. As a control, a parallel extraction was made of agar on which no fungus growth had occurred.

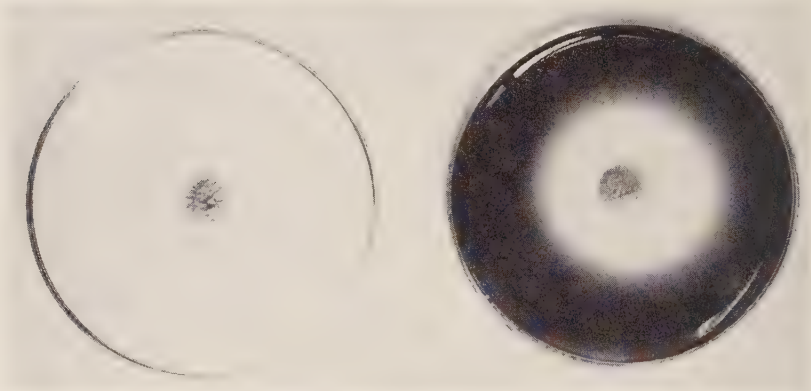

**Fig. 7. — Amylase activity of *Rhynchosporium secalis*. Left, three-week-old culture on lima bean agar. Diameter increase has ceased. Right, companion culture flooded with Lugol's iodine solution after three-weeks growth. Note clear zone surrounding fungus where starch medium has been changed to dextrin.**

The filtrate was divided into three aliquots. One was boiled to test for heat inactivation of the enzyme. To each of the three was added 60 ml of a standardized "soluble" starch solution. One of the unboiled aliquots was adjusted to pH 7, and the other to pH 4.9. The extract from unused agar was combined with starch solution and adjusted to pH 7. Each sample then had a turbid appearance in transmitted light.

A Bausch and Lomb Spectronic 20 colorimeter was used as a nephelometer to measure the transmission of light at a standard wave length. As the enzyme acted, the transmission increased. Readings on each sample were made each half hour for $3\frac{1}{2}$ hours. A distilled water blank and aliquots of the standard starch suspension at pH 7 and 4.9 were used as standards.

Figure 8 shows the data yielded by this study. In it the plotted straight lines represent (a) standard starch suspension, (b) standard suspension with boiled enzyme extract, (c) distilled water, and (d) extract from unused agar. The curves show that the greatest net change in light transmission and thus presumably in enzyme activity occurs 1 to 2 hours after mixing. Activity of the enzyme is greater at pH 7 than at pH 4.9. The differences among the isolates are not striking.

This test proved that a starch hydrolyzing enzyme was present in the cleared zone in advance of the fungus mycelium and that the enzyme was produced by the fungus. To determine what had happened to the starch, the solutions resulting after hydrolysis was completed were centrifuged to remove any remaining starch particles. At half-hour intervals, the following amounts of ethanol were added to a

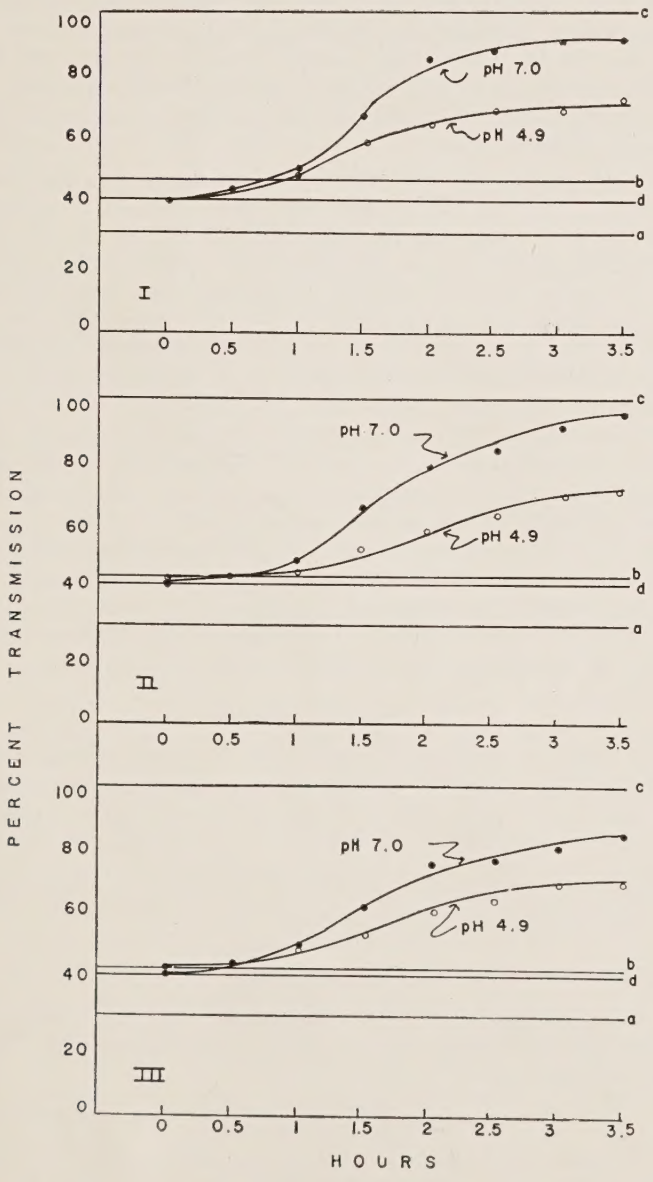


Fig. 8. — Percentage transmission of light through starch suspensions after different periods of exposure to enzyme derived from *Rhynchosporium secalis*.

90 ml sample: 22.5 ml, 37.5 ml, 75 ml, and 225 ml. The final solution was 80 per cent alcohol. After this fractional precipitation was completed, the 80 per cent alcohol sample was filtered and the filtrate distilled under vacuum until only a few milliliters remained. This remainder was made up to 20 ml with distilled water and used for chromatographic analysis.

To each paper strip was pipetted 6 lambda on one spot and the spots dried. The chromatograms were developed in a solution of butanol: pyridine: water in the ratio of 6:4:3 by volume. Following the procedure of Meyerback and Willstaedt (*7*) the column was allowed to run for 24 hours and the paper was then dried. After the method of Trevelyan and associates (*14*), the paper was immersed in silver nitrate, in acetone, dried, and sprayed with 0.5 N NaOH in ethanol. Excess silver oxide was removed by immersion in 6 N $NH_4$ OH; the paper was washed for 1 hour and dried.

Glucose checks had been spotted on the chromatograms, and examination showed these to be the only evident spots on the papers. No other sugars were present according to this analysis. From this test and the previous one showing greatest activity of the enzyme at pH 7 it was concluded that the active agent was an alpha-amylase or dextrinizing starch enzyme. It was further concluded that this enzyme is secreted by the fungus, that it pervades the agar ahead of the fungus growth. This advance dextrinization of the medium may play a role in the determinate growth of the organism in culture.

### E. Nuclear Number

A number of studies were made with the three isolates to determine if variation observed among them might be a heterokaryotic phenomenon associated with a multinucleate condition. Such work has since been reported by other workers (*7 and 12*) and it is necessary to say here only that in stained preparations, and in live ones observed by phase contrast microscopy, only 1 nucleus per cell was seen. This corroborates the work cited.

### F. Conidial Size

Conidia were harvested from 18-day-old test-tube cultures of the 3 isolates and 500 conidia of each were measured for size. The following data resulted:

| Isolate | Average Length (u) | Average Width (u) |
|---|---|---|
| I | 17.3 (14.0 to 24.5) | 3.5 (2.8 to 4.2) |
| II | 14.8 (11.9 to 18.9) | 3.5 (2.8 to 4.2) |
| III | 15.1 (11.9 to 21.7) | 3.5 (2.8 to 4.2) |

Obvious differences were observed that might serve as morphologic bases for differentiation of isolates. This evidence, however, was not as good as pathogenic specialization in classifying this organism, and so the races have been differentiated only through pathogenicity. It should be noted that the conidia of isolate I possessed the well-described apical beak. Those of II and III were more blunt.

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